

# REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words)  The activities of the Children's Hospital of Pittsburgh Histocompatibility Center can be classified under three major categories: 1) the repository service; 2) the molecular typing service; and 3) the research activities aimed at improving the technical support on which the previous two services are working. This year in the Repository, where blood samples from volunteers as bone marrow donors are collected from more than 125 recruiting centers nationwide, 165,203 samples were processed. We stored 495,609 vials in three sets of freezers (one sample per donor each freezer), while 216,712 were sent out to other Typing Laboratories for molecular HLA typings. In our laboratories, 10,626 typings were performed with a very high Q.C. standard, as monitored by National Marrow Donor Program. Our Center's research branch synthesized all the oligonucleotides to be used as PCR primers or labelled probes to perform molecular HLA typing. DNA sequencing was also performed when hybridization results were not optimal. Alternatives for storing blood samples (e.g., blood spots on filter paper) or for enhancing typing capabilities (e.g., microchip technology to expedite PCR procedures) were successfully implemented or improved to the point of justifying their future implementation.				
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# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-98-1-0908

Total Samples Received, Stored and Shipped from the Repository from 10/01/98 to 09/30/99

<b>SAMPLES</b>	<b>NUMBER (October 1998)</b>	<b>NUMBER (November 1998)</b>	<b>NUMBER (December 1998)</b>
Stored	15,353	15,878	15,620
Shipped (DR - Class II)	10,003	11,840	12,922
Shipped (AB - Class I)	2,816	3,184	2,800
Shipped (Q.C. - Serology)	---	65	195
Destroyed	1,333	515	0
<b>SAMPLES</b>	<b>NUMBER (January 1999)</b>	<b>NUMBER (February 1999)</b>	<b>NUMBER (March 1999)</b>
Stored	11,997	11,493	19,903
Shipped (DR - Class II)	9,335	7,287	10,628
Shipped (AB - Class I)	2,900	4,206	10,494
Shipped (Q.C. - Serology)	149	130	130
Destroyed	1,361	816	2,432

<b>SAMPLES</b>	<b>NUMBER (April 1999)</b>	<b>NUMBER (May 1999)</b>	<b>NUMBER (June 1999)</b>
Stored	18,510	13,184	14,427
Shipped (DR - Class II)	9,555	10,584	11,456
Shipped (AB - Class I)	9,235	10,522	8,550
Shipped (Q.C. - Serology)	129	102	127
Destroyed	649	569	922
<b>SAMPLES</b>	<b>NUMBER (July 1999)</b>	<b>NUMBER (August 1999)</b>	<b>NUMBER (September 1999)</b>
Stored	10,339	8,660	9,839
Shipped (DR - Class II)	13,334	14,902	14,624
Shipped (AB - Class I)	7,200	9,155	8,153
Shipped (Q.C. - Serology)	0	0	0
Destroyed	2,219	1,954	913

- Stored samples (Multiply number stored x 3 because of triplicate aliquots)
- Shipped samples (Separated by AB and DR samples)
- Shipped samples (Q.C. - Serology for NMDP Special Study 11/98 - 6/99)
- Destroyed because no longer eligible, so that blood sample must be removed from NMDP list.

# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-98-1-0908

Total Samples Typed and Results Sent from 10/01/98 to 09/30/99

	Total	Priority 1	Priority 2	Priority 3	No Makes	Navy
<b>1998</b>						
October	819	103	714	2	0	0
November	1,000	185	806	7	2	0
December	759	163	588	8	0	0
<b>1999</b>						
January	869	143	720	4	2	0
February	969	98	862	6	3	0
March	1,105	70	1,034	0	1	0
April	608	227	375	6	0	0
May	1,021	123	894	3	1	0
June	878	175	699	4	0	0
July	962	76	886	0	0	0
August	819	90	714	12	3	0
September	817	68	746	3	0	0

# CHILDREN'S HOSPITAL OF PITTSBURGH HISTOCOMPATIBILITY CENTER

AWARD NUMBER: N00014-98-1-0908

Research Completed from 10/01/98 to 10/31/99

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Taqman Probes:	Miscellaneous Research Projects:
Oct '98	38	145	0	Continued evaluation of various purification conditions for doubly-labelled fluorescent probes were examined: TAMRA labelling conditions and HPLC parameters were designed to achieve better signal to noise ratios.
Nov '98	48	184	1	Manipulations of HPLC conditions and TAMRA labelling conditions were examined for better signal to noise ratios involving doubly-labelled fluorescent probes, along with various purification protocols.
Dec '98	62	108	12	<p>Multiplex reactions containing DQ-specific reactions and internal reactions were achieved using two differently labelled fluorescent probes.</p> <p>Continued evaluation of Taqman technology and doubly-labelled fluorescent probes for HLA typing.</p> <p>Synthesis and cleavage of 5 peptides on ABI Synergy 432A Peptide Synthesizer.</p> <p>Performed quarterly Quality Control and annual preventive maintenance on all automated equipment.</p>
Jan '99	32	141	6	Evaluation of 96-well plate format for purification of samples following cycle sequencing.
Feb '99	35	192	0	Evaluation and implementation of Amersham-Pharmacia Biotech Autoseq G-50 columns for sample purification following cycle sequencing.

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Taqman Probes:	Miscellaneous Research Projects:
Mar '99	64	97	0	<p>Evaluation and implementation of Amersham-Pharmacia Biotech Microspin G-25 columns for purification of oligonucleotides following synthesis and deprotection.</p> <p>Performed quarterly Quality Control on all automated equipment.</p>
Apr '99	77	184	2	<p>Replaced HPLC column and updated instrument to optimally perform fluorescent-labelled oligonucleotide purification. Performed quality control on updated instrument.</p>
May '99	53	238	4	<p>Purchased and installed one new G3 Macintosh computer required for updated software for DNA sequencers.</p> <p>Installed new data collection and sequencing software.</p> <p>Began troubleshooting communication difficulties between new sequencing collection software and the instrument.</p>
Jun '99	72	205	2	<p>Performed quarterly Quality Control on all automated equipment.</p>
July '99	64	408	0	<p>Continued to trouble-shoot and evaluate sequencing hardware and software.</p> <p>Purchased and installed a second G3 Macintosh from ABI to compare internal hardware components.</p>
Aug '99	52	144	8	<p>Installation, optimization and evaluation of ABI HLA typing kit incorporating sequence-based typing strategies. Typing performed on 15 patient blood samples and results compared to SSOP typing results for confirmation.</p>
Sep '99	127	129	0	<p>Resolved communication conflicts with new Macintosh computer and DNA sequencer. Both new computers and instruments functioning optimally using updated software. Passed quality control check with known samples.</p>

Month/ Year:	Oligonucleotides Synthesized:	ABI/PE Sequencing:	Taqman Probes:	Miscellaneous Research Projects:
Oct '99	51	133	0	<p>Performed quarterly Quality Control and annual preventive maintenance on all automated equipment.</p> <p>Installed new ABI Genescan software for use on the DNA sequencers.</p> <p>Began and completed trouble-shooting of data collection software for fragment analysis on DNA sequencers.</p>